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Hypertension and renal injury in experimental polycystic kidney disease

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Background. Hypertension accelerates renal failure in autosomal dominant polycystic kidney disease (ADPKD), and evidence suggests a role for the renin-angiotensin system (RAS) in the functional and structural changes. To explore the hypothesis that RAS adaptations contribute to disease progression, we examined RAS activity and the long-term consequences of antihypertensive drugs, which suppress (enalapril) or stimulate (hydralazine) the RAS, in experimental polycystic kidney disease.

Methods. Studies were conducted in male heterozygous cystic Han:SPRD rats (Cy/+) and in unaffected littermates (controls). In protocol 1, either angiotensin II (Ang II), enalaprilat, or saline vehicle was acutely infused into cystic and control rats, which were aged 10 to 12 weeks. The mean arterial pressure (MAP), glomerular filtration rate (GFR), and renal plasma flow (RPF) were measured at baseline and after an infusion of test substances. In protocol 2, cystic rats received chronic therapy with either enalapril, hydralazine, or no therapy for 10 to 12 weeks of age and then underwent renal function and RAS studies. In protocol 3, similar cohorts were followed for 40 weeks to assess the effects of therapy on blood pressure, proteinuria, serum creatinine, RAS parameters, and renal morphology.

Results. In protocol 1, cystic rats had massive kidneys, slightly elevated blood pressure, and profound renal vasoconstriction and reduced GFR. Ang II induced similar changes in MAP and renal function in control and cystic rats. Enalaprilat induced little effect on MAP but more striking increases in GFR and RPF in cystic rats. In protocol 2, at 10 weeks of age, enalapril was superior in preserving renal function, but neither drug limited the expansion of the tubulointerstitium. In protocol 3, at 40 weeks of age, both drugs ameliorated the increase in serum creatinine, although only enalapril reduced proteinuria and kidney size.

Conclusions. In polycystic rats, acute RAS suppression markedly ameliorates renal dysfunction. However, although chronic enalapril and hydralazine protect against the loss of renal function, only enalapril limits renal growth and proteinuria, and neither significantly limits tubulointerstitial fibrosis. The long-term studies give clear support to the importance of blood pressure control, *per se*, but only partial support to the importance of the particular agent used. As in clinical studies, angiotensin-converting enzyme inhibition may be less beneficial in ADPKD than in renal diseases characterized by predominant glomerular injury.

Key words: PKD, angiotensin, hydralazine, interstitial fibrosis, progressive renal disease.

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One of the most common hereditary diseases, autosomal dominant polycystic kidney disease (ADPKD), represents approximately 10% of the cases of end-stage renal disease in the United States. Clinical and experimental studies have provided a detailed record of the clinical, physiological, and molecular manifestations of ADPKD [1–4], and two of the genes that are associated with ADPKD have been isolated [5, 6]; however, the mechanisms that result in cyst formation and renal failure are complex. Complicating this issue are the long and variable clinical course to renal failure and, until recently, the lack of a comparable experimental model of ADPKD.

An important factor that accelerates renal failure in ADPKD is hypertension [3, 7–10]. Hypertension occurs prior to a loss of renal function in 50 to 75% of patients with ADPKD. In addition, renal size has been correlated with hypertension [11], suggesting a relationship between hypertension and cyst growth. Angiographic analysis of polycystic kidneys reveals a marked distortion of the renal vascular tree and cystic compression of surrounding tissue, suggesting that vascular attenuation and parenchymal ischemia may be contributing to the generation of hypertension and progression of renal failure in this disorder [12]. Findings of low renal plasma flow (RPF), high renal vascular resistance (RVR), and elevation of filtration fraction [7, 13, 14], as well as severe sclerosis of the preglomerular vessels even early in the course of the disease [15], are further evidence for relative intrarenal vasoconstriction in ADPKD.

The hemodynamic pattern described earlier in this

article is suggestive of an important influence of the renin-angiotensin system (RAS). That the RAS may be involved in the pathogenesis of hypertension and renal injury in ADPKD is further suggested by observations of: higher values for plasma renin activity (PRA) in hypertensive ADPKD patients [16]; attenuated hemodynamic responses to exogenous angiotensin II (Ang II) infusion [17], suggesting down-regulation of Ang II receptors; and normalization of RVR and filtration fraction with angiotensin-converting enzyme inhibition (ACEI) [14, 18]. Additionally, the failure of RAS suppression in hypertensive subjects with ADPKD, despite evidence of increased circulating volume, suggests that maladaptive stimulation of the RAS may be a central feature in these derangements [9, 10, 16].

In the current study, we examined the hypothesis that important adaptations of the RAS occur in experimental ADPKD and that these effects result in a primary and maladaptive defect in vascular regulation that ultimately contributes to disease progression. We studied several aspects of RAS biochemistry and physiology, as well as the long-term consequences of antihypertensive therapy, which suppresses (enalapril) or stimulates (hydralazine) the RAS, in the Han:SPRD model of experimental polycystic kidney disease.

METHODS

The autosomal dominant polycystic kidney disease model

These studies were conducted in male Han:SPRD rats, which were raised in a breeding colony propagated from breeding pairs kindly provided by Dr. Benjamin Cowley (University of Kansas Medical Center, Kansas City, KS, USA). Heterozygous cystic rats (Cy/+) and unaffected littermate control rats (+/+) were studied. All rats were fed standard rat chow (Rodent Laboratory Chow 5001; Purina Mills, Richmond, IN, USA) *ad libitum* and had free access to water. These studies were approved by the Portland Veterans Affairs Institutional Animal Care and Use Subcommittee.

Protocol 1: Acute treatment studies

To examine the systemic hemodynamic and renal functional roles of the RAS in polycystic kidney disease, acute intravenous infusions of either Ang II, enalaprilat, or normal saline vehicle (time controls) were administered to cystic and control rats (age 10 to 12 weeks, N = 7 to 9 per group). Following surgical preparation for functional studies and after a one-hour period of equilibration, all rats underwent two timed (20 min) urine collections for the determination of flow rates, inulin, and paraaminohippuric acid (PAH). Blood was obtained simultaneously for measurement of hematocrit (Hct), inulin, and PAH. Glomerular filtration rate (GFR) from inulin clearance and RPF from PAH clearance were determined using standard formulas. The mean arterial pressure (MAP) was also recorded during each collection period, and RVR was calculated from measurements obtained. Following these baseline measurements, rats received either normal saline, Ang II (0.2 μg/kg/min), or enalaprilat (2 mg bolus, then a continuous infusion of 2 mg/hr). Following a 30-minute period of equilibration, functional measurements were repeated during the experimental period. After the experiments, blood was taken for the measurement of the plasma renin concentration (PRC).

Protocol 2: Chronic treatment studies (10 weeks)

To examine the chronic functional and morphologic effects of manipulations of the RAS, additional groups of cystic rats were given either no therapy, enalapril (50 mg/liter in the drinking water), or hydralazine (160 mg/liter), beginning at weaning (age of three weeks). Control rats were given tap water. At ages 10 to 12 weeks, six to nine rats per group underwent surgical preparation and baseline functional studies as described in protocol 1. After the hemodynamic measurements, kidneys were prepared for morphologic studies as described later in this article.

Protocol 3: Chronic treatment studies (40 weeks)

Rats were prepared and treated with either hydralazine or enalapril as described in protocol 2. These groups of rats (N = 6 to 10/group) were followed up to 40 weeks of age, with serial measurements of tail cuff blood pressure and 24-hour measurements of urinary protein excretion. At 40 weeks, rats were sacrificed, with blood and tissue obtained for measurements of plasma renin concentration (PRC), serum creatinine (S_{Cr}), and plasma and renal Ang II levels, and kidneys were prepared for morphologic studies as described later in this article.

Renal function studies

Rats were anesthetized with Inactin (100 mg/kg intraperitoneally) and placed on a thermoregulated table. The left femoral artery was cannulated, and a baseline sample of blood was collected for determination of Hct and inulin and PAH blanks. This arterial catheter was used for subsequent blood sampling and for the estimation of MAP via an electronic transducer connected to a direct writing recorder. After tracheostomy, bilateral internal jugular catheters were inserted for infusions of rat serum, and 10% inulin with 1.0% PAH in saline (1.2 ml/hr). To adjust for reduced renal clearances, cystic rats were given 6% inulin with 0.6% PAH in saline. A femoral venous catheter was used for the delivery of study drugs, and the left ureter was catheterized for urine collections. To maintain euvolemia, rat serum was infused at 0.1 ml/min for a total equal to 1% of the body weight,

followed by a reduction in infusion rate to 0.42 ml/hr, to maintain a constant Hct.

Biochemical studies

For the calculation of GFR, inulin concentrations in plasma and urine were determined by the macro-anthrone method. RPF was determined by PAH clearance, with PAH concentrations in plasma and urine determined by colorimetric methodology. PRC was measured by incubating 100 μl of rat plasma with 100 μl rat anephric plasma and 400 μl of 0.2 M maleate buffer, pH 6.0, at 37° for one hour. Appropriate dilutions of rat plasma samples were made using Tris buffer. The generation of Ang I was then determined by radioimmunoassay using commercially available reagents (New England Nuclear, Boston, MA, USA). Ang II was measured using the methods of Fox et al [19]. Blood was collected by cardiac puncture into a prechilled syringe, with 1 ml being rapidly placed into 9 ml cold methanol. Kidneys were rapidly homogenized in 10 ml of cold methanol. Samples were extracted using a phenyl-bonded SPE column (Bond-Elut; Varian, Harbor City, CA, USA). Ang II levels were quantitated by radioimmunoassay, using rabbit anti-Ang II antisera (Peninsula, Belmont, CA, USA), monoiodinated ^{125}I-labeled Ang II (Amersham, Arlington Heights, IL, USA), and Ang II standards (Sigma, St. Louis, MO, USA). Renal Ang II levels were expressed as fmol Ang II per mg kidney (wet) weight and also as fmol/mg protein. Renal protein content was measured using a bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, USA), using bovine serum albumin (BSA) as the standard. Urinary protein was measured by precipitation with 3% sulfosalicylic acid.

Morphometric studies

Renal morphometry was assessed quantitatively using the point counting method. Kidneys were prepared by 10% formalin fixation, and slides were stained using hematoxylin and eosin staining. Subcapsular cortical fields were assessed using a rigid protocol for random positioning. Cystic kidneys were assessed at low power (×100) to avoid sampling variations produced by massive cystic dilation. Control rats were assessed at ×400. Cysts, which were distinguished from tubules by their flattened epithelial surfaces, were avoided and not counted. Results are expressed as a percentage of the total area.

Statistics

Values are reported as means ± SEM. Statistical analysis was performed by paired *t*-test (for studies before and after an intervention) or by paired *t*-test or analysis of variance followed by computation of modified *t*-values according to the method of Bonferroni (for multiple groups), as appropriate. Values that were not normally distributed were analyzed by nonparametric methods. Statistical significance was defined as $P < 0.05$.

Table 1. Baseline systemic and renal parameters at 10 weeks of age

	Control rats ($N = 23$)	Cystic rats ($N = 24$)	P value
BW *g*	335 ± 8	335 ± 7	>0.05
LKW *g*	1.55 ± 0.05	4.53 ± 0.13	<0.001
LKW/100 g BW	0.46 ± 0.01	1.37 ± 0.04	<0.001
MAP *mm Hg*	121 ± 2	133 ± 2	<0.001
GFR *ml/min*	1.56 ± 0.05	0.76 ± 0.03	<0.001
RPF *ml/min*	5.89 ± 0.24	2.61 ± 0.15	<0.001
FF	0.27 ± 0.01	0.30 ± 0.01	<0.05
RVR *mm Hg/(ml/min)*	12.5 ± 0.6	33.8 ± 2.2	<0.001

Abbreviations are: BW, body weight; LKW, left kidney weight; MAP, mean arterial pressure; GFR, glomerular filtration rate; RPF, renal plasma flow rate; FF, filtration fraction; RVR, renal vascular resistance.

RESULTS

Protocol 1

The results of protocol 1, in which acute hemodynamic responses to RAS stimulation or suppression were determined in rats aged 10 weeks, are depicted in Tables 1 and 2 and Figure 1. Because baseline values in the various control and cystic groups were comparable, they were pooled for analysis (Table 1). Body weights were comparable, whereas values for both the left kidney weight and the kidney/body weight ratio were markedly increased (approximately threefold) in the cystic rats as compared with noncystic controls. Although the Han:SPRD rats do not develop severe hypertension, they were slightly hypertensive as compared with the unaffected control rats. By 10 weeks of age, renal functional impairment was apparent, with markedly reduced values for GFR and RPF. The marked vasoconstriction was associated with significant elevations in filtration fraction and in RVR (Table 1).

The effects of acute infusion of Ang II, enalaprilat, and saline vehicle are depicted in Table 2 and Figure 1. Saline vehicle had no significant effect on any parameter in either group. In noncystic control rats, Ang II induced a significant increase in MAP. GFR was relatively unchanged, whereas RPF was markedly reduced (−46 ± 7% as compared with baseline; Fig. 1). These changes were associated with marked increases in filtration fraction and in RVR (+140 ± 27%). In cystic rats receiving Ang II, the systemic pressor response was similar to that in noncystic controls. GFR was not affected. The decrement in RPF and increment in RVR were greater than in the control rats, whereas a similar percentage of reductions in RPF (−46 ± 6%) and increments in filtration fraction and RVR (+134 ± 27%) was seen.

Enalaprilat induced modest, but not significant, reductions in MAP in both control and cystic groups. In control

Table 2. Effects of angiotensin II and enalaprilat on systemic and renal hemodynamic function

Group	Period	MAP *mm Hg*	GFR *ml/min*	RPF *ml/min*	FF	RVR *mm Hg/(ml/min)*	PRC *ng Ang I/ml/hr*
C + NS ($N = 7$)	1	120 ± 2	1.40 ± 0.09	5.08 ± 0.37	0.28 ± 0.01	14.3 ± 1.3	77 ± 18
	2	118 ± 3	1.44 ± 0.09	5.31 ± 0.37	0.27 ± 0.01	14.3 ± 1.3	
	Δ	−2 ± 2	+0.04 ± 0.05	+0.23 ± 0.33	−0.01 ± 0.01	−0.7 ± 1.1	
Cystic + NS ($N = 7$)	1	134 ± 3	0.70 ± 0.06	2.31 ± 0.26	0.31 ± 0.02	39.1 ± 4.3	9 ± 2[c]
	2	129 ± 5	0.75 ± 0.06	2.41 ± 0.27	0.32 ± 0.01	36.9 ± 4.6	
	Δ	−6 ± 3	+0.05 ± 0.04	+0.09 ± 0.11	+0.01 ± 0.01	−2.2 ± 2.5	
C + Ang II ($N = 8$)	1	119 ± 4	1.50 ± 0.06	5.71 ± 0.22	0.27 ± 0.02	12.4 ± 0.8	10 ± 1[b]
	2	139 ± 4[a]	1.37 ± 0.14	3.03 ± 0.27[a]	0.45 ± 0.01[a]	28.7 ± 2.2[a]	
	Δ	+20 ± 4[a,b]	−0.13 ± 0.13	−2.68 ± 0.42[a,b]	+0.18 ± 0.01[a,b]	+16.2 ± 2.8[a,b]	
Cystic + Ang II ($N = 8$)	1	134 ± 2	0.85 ± 0.06	2.87 ± 0.30	0.31 ± 0.02	30.2 ± 3.9	4 ± 1[b,c]
	2	150 ± 5[a]	0.71 ± 0.05	1.48 ± 0.17[a,b]	0.51 ± 0.05[a,b]	67.4 ± 7.7[a,b]	
	Δ	+17 ± 4[a,b]	−0.14 ± 0.06	−1.39 ± 0.24[a,b,c]	+0.20 ± 0.04[a,b]	+37.2 ± 7.4[a,b,c]	
C + Enal ($N = 7$)	1	124 ± 3	1.77 ± 0.08	6.77 ± 0.43	0.27 ± 0.02	11.1 ± 0.7	403 ± 111[b]
	2	119 ± 4	1.88 ± 0.14	7.98 ± 0.24[a]	0.24 ± 0.01[a]	9.1 ± 0.4	
	Δ	−5 ± 4	+0.11 ± 0.10	+1.21 ± 0.48[a]	−0.03 ± 0.01[a]	−2.0 ± 0.5	
Cystic + Enal ($N = 9$)	1	132 ± 3	0.73 ± 0.05	2.60 ± 0.20	0.29 ± 0.02	33.0 ± 2.8	45 ± 15[b,c]
	2	129 ± 4	1.09 ± 0.09	3.77 ± 0.40[a]	0.30 ± 0.01	23.9 ± 2.7	
	Δ	−4 ± 2	+0.36 ± 0.07[a,b]	+1.17 ± 0.32[a]	+0.01 ± 0.01	−9.1 ± 1.8[a]	

Period 1 = baseline. Period 2 = experimental.

Abbreviations are: C, control rats; NS, normal saline; Ang II, angiotensin II; Enal, enalapril; Δ, difference between Periods 1 and 2; PRC, plasma renin concentration; other abbreviations as in Table 1.

[a] $P < 0.05$ vs. baseline (Period 1)

[b] $P < 0.05$ vs. NS group or change in NS group

[c] $P < 0.05$ vs. change in corresponding controls

Differences between control and cystic rats in baseline periods in each group were similar to those in Table 1, and are not shown.

rats, GFR was unaffected, whereas RPF increased. The filtration fraction fell slightly, and RVR was numerically, but not statistically, reduced slightly as well. Of note, renal hemodynamic responses to enalaprilat were much more striking in the cystic rats, particularly when expressed as a percentage change from baseline. Prominent increases in both GFR (+52 ± 11%) and RPF (+46 ± 14%) were noted (Fig. 1). Filtration fraction was unchanged, whereas RVR fell significantly (−28 ± 5%). Thus, although acute hemodynamic responses to exogenous Ang II were not different in the cystic rats, the effects of acute blockade of Ang II formation with enalaprilat were markedly enhanced.

Of note, the aforementioned hemodynamic responses occurred in the absence of systemic RAS activation. In fact, values for PRC were consistently reduced in the cystic rats, even with the stimuli of anesthesia and surgery (Table 2). In control rats, PRC values were appropriately reduced by Ang II infusion and were increased by enalaprilat. Directionally similar responses occurred in the cystic rats, but values under all conditions were significantly lower than those noted in control animals.

Protocol 2

The results of studies of short-term treatment with hydralazine and enalapril are summarized in Table 3. Body weights were similar in all groups. As compared with the noncystic controls, cystic rats again exhibited slightly higher blood pressures (though not significantly) and renal vasoconstriction with elevated RVR. Blood pressure was equivalently and significantly reduced by enalapril and hydralazine. Both antihypertensive regimens had beneficial effects on renal hemodynamic function numerically, but enalapril afforded superior improvement. As compared with untreated cystic rats, only the rats receiving enalapril had statistically higher values for GFR and RPF. Hydralazine-treated rats had lower values for filtration fraction, and both groups had significant reductions in RVR.

Although still enlarged as compared with normal animals, kidneys in both treated groups were smaller than those in the untreated cystic rats, presumably reflecting a diminished cyst burden. However, despite the preservation of GFR with enalapril, morphologic studies did not find significant preservation of renal structure in either group. Morphometric analysis revealed, as expected, prominent increases in the percentage of the kidney taken up by tubular lumens and the interstitium in the untreated cystic rats. Neither antihypertensive drug significantly limited the tubulointerstitial expansion.

Protocol 3

Results of long-term (up to age 40 weeks) studies with enalapril and hydralazine are summarized in Table 4. At the final systolic blood pressure determination, untreated cystic rats were clearly hypertensive, and blood pressures were equivalently reduced with the two antihypertensive drugs. Time-averaged values for blood pressure, span-

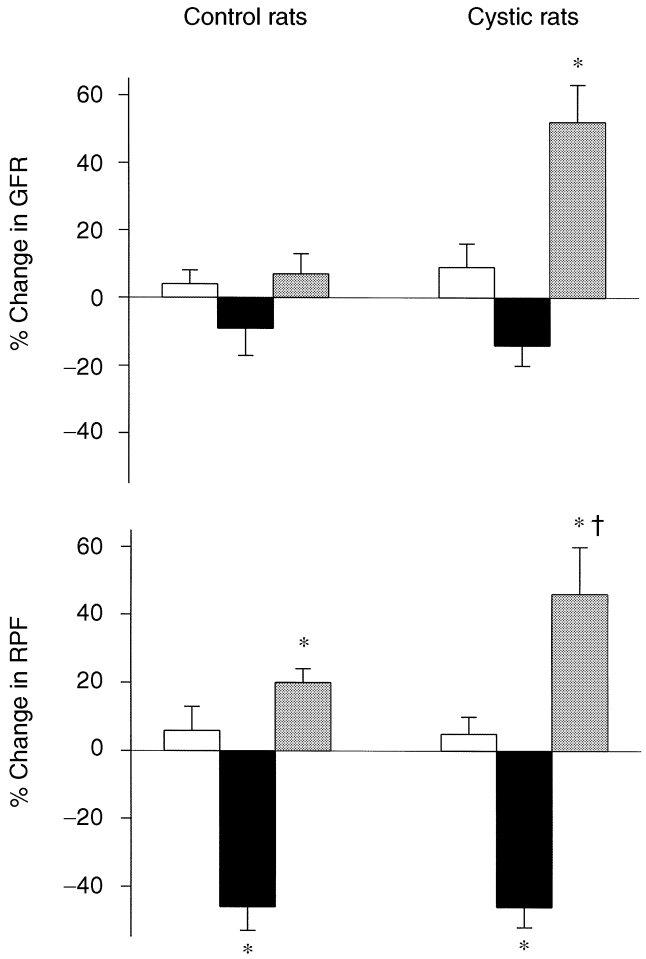

Fig. 1. Effects of saline vehicle (□), angiotensin II (Ang II; ■), and enalapril (▩) in control and cystic rats, expressed as a percentage change in glomerular filtration rate (GFR; *A*) and renal plasma flow rate (RPF; *B*) from baseline values. $*P < 0.05$ vs. baseline; $\dagger P < 0.05$ vs. change in control rats.

ning the entire treatment period, revealed the same pattern. Cystic rats developed moderate and significant proteinuria, which was attenuated by enalapril but not hydralazine. Body weights were slightly reduced in the enalapril-treated rats but were comparable in the other groups. Renal enlargement was again apparent in the untreated rats. Values for left kidney weight were markedly lower in the enalapril group, although the difference was less apparent when factored for body weight. Gross heart weights and heart/body weight ratios were significantly increased in the untreated cystic rats and were reduced only with enalapril. Renal function, as assessed by serum creatinine, was markedly impaired in the cystic rats. Of note, both hydralazine and enalapril treatment ameliorated renal dysfunction so that serum creatinine elevations were markedly attenuated.

Values for PRC were markedly reduced in the cystic rats, as compared with noncystic controls. As expected, both enalapril and hydralazine increased PRC values. Blood Ang II levels tended to follow the patterns suggested by the PRC values and the known effects of these drugs. Blood Ang II levels were low in the cystic rats and in cystic rats treated with enalapril, although values in hydralazine rats were higher and closer to those in control rats. When expressed per gram of wet kidney weight, renal Ang II levels were comparable in control and untreated cystic rats and were reduced with enalapril but not hydralazine. Given the markedly different kidney weights, we also examined renal Ang II factored per mg renal protein. When so factored, renal Ang II levels were slightly reduced in the cystic rats, clearly reduced in the enalapril-treated rats, and markedly increased in the hydralazine-treated cystic rats.

Morphologic patterns tended to follow those seen at 10 weeks: marked expansion of the tubulointerstitium in the cystic rats, without significant modification by antihypertensive therapy. Although differences in the percentage of morphologic structures did not differ, the lower kidney weights in the enalapril-treated rats most likely reflected diminution in total cyst burden.

DISCUSSION

These studies were conducted in the Han:SPRD model of ADPKD, which has proven highly useful in reflecting the major pathophysiological features of the human disease [20–22]. In this model, homozygous affected animals develop rapid renal failure and do not survive past the first month. The heterozygotes grow normally and survive for approximately 8 to 12 months, while developing the major renal manifestations of the disease: progressive renal enlargement caused by cyst formation, tubulointerstitial fibrosis, and impairment of GFR [20].

When studied at 10 weeks of age, the male Han:SPRD rats were already manifesting the major features of the disease. Although hypertension was less severe than that seen in some other models of progressive renal disease, it was generally present, confirming findings in previous studies in this model [22, 23] and in humans with the disease [7–10]. Blood pressure values in the unaffected littermate controls were within the normal range and were similar to those in the parent Sprague-Dawley strain, confirming that the blood pressure elevation was associated with the polycystic disease. Clearance studies confirmed profound renal vasoconstriction, with reduced values for GFR and RPF, and elevated values for filtration fraction (FF) and RVR; these findings are similar to those noted in young adults with relatively early ADPKD [8, 13, 14, 24, 25].

The mechanisms of this intense renal vasoconstriction remain incompletely defined. Renal parenchymal ischemia, resulting from compression induced by massive cystic enlargement, has been postulated to play a role, per-

Table 4. Effects of enalapril and hydralazine on the kidney at 40 weeks of age

	Control ($N = 6$)	Cystic ($N = 9$)	Cystic/enal ($N = 10$)	Cystic/hydral ($N = 9$)
Final SBP *mm Hg*	113 ± 4	158 ± 9[a]	116 ± 4[b]	108 ± 3[b]
Average SBP *mm Hg*	121 ± 3	130 ± 6[a]	110 ± 2[a,b]	107 ± 2[a,b]
$U_{prot}V$ *mg/day*	4 ± 1	54 ± 12[a]	24 ± 2[a,b]	78 ± 18[a,b]
BW *g*	480 ± 6	483 ± 14	444 ± 10[b]	458 ± 6
LKW *g*	1.68 ± 0.03	3.65 ± 0.43[a]	2.14 ± 0.31[b]	3.53 ± 0.24[a,c]
LKW/100 g BW	0.35 ± 0.01	0.77 ± 0.10[a]	0.64 ± 0.03	0.77 ± 0.05[a]
HW *g*	1.47 ± 0.03	1.73 ± 0.07[a]	1.38 ± 0.05[b]	1.69 ± 0.05
HW/100 g BW	0.31 ± 0.01	0.36 ± 0.02[a]	0.31 ± 0.01[b]	0.37 ± 0.01[a,c]
Serum Cr *mg/dl*	0.69 ± 0.4	2.01 ± 0.51[a]	1.03 ± 0.05[b]	0.98 ± 0.04[b]
PRC *ng Ang I/ml/hr*	22 ± 3	4 ± 0.2[a]	64 ± 13[a,b]	43 ± 17[a,b]
Blood Ang II *fmol/ml*	19 ± 3	6 ± 2[a]	10 ± 3	16 ± 2[b]
LK Ang II *fmol/g*	124 ± 11	79 ± 28	28 ± 5[a,b]	109 ± 28[c]
LK Ang II *fmol/mg prot*	1.42 ± 0.17	0.97 ± 0.13[a]	0.66 ± 0.17[a]	3.08 ± 0.97[a,b,c]
% Tubules (cells)	71.9 ± 4.7	22.2 ± 4.3[a]	21.5 ± 2.1[a]	26.2 ± 2.3[a]
% Tubules (lumen)	7.5 ± 0.7	30.2 ± 4.4[a]	28.3 ± 2.3[a]	22.0 ± 1.7[a]
% Tubules (total)	79.3 ± 4.5	52.4 ± 1.3[a]	49.8 ± 2.0[a]	48.2 ± 2.9[a]
% Glomeruli	7.2 ± 1.4	4.8 ± 0.5[a]	4.5 ± 0.4[a]	4.0 ± 0.4[a]
% Vessels	0.3 ± 0.1	1.3 ± 0.3[a]	0.7 ± 0.1	1.4 ± 0.3[a]
% Interstitium	8.7 ± 1.8	41.5 ± 1.3[a]	45.1 ± 2.0[a]	46.5 ± 3.2[a]

Abbreviations are: SBP, systolic blood pressure; $U_{prot}V$, 24-hour urinary protein excretion; HW, heart weight; Cr, creatinine; PRC, plasma renin concentration; other abbreviations as in Tables 1–3.

[a] $P < 0.05$ vs. controls

[b] $P < 0.05$ vs. cystic rats

[c] $P < 0.05$ vs. cystic/enal rats

Table 3. Effects of enalapril and hydralazine on the kidney at 10 weeks of age

	Control rats ($N = 8$)	Cystic rats ($N = 6$)	Cystic/enal ($N = 9$)	Cystic/hydral ($N = 9$)
BW *g*	325 ± 8	330 ± 6	313 ± 10	303 ± 10
MAP *mm Hg*	122 ± 2	132 ± 6	107 ± 2[a,b]	111 ± 3[a,b]
GFR *ml/min*	1.82 ± 0.05	0.78 ± 0.08[a]	1.28 ± 0.08[a,b]	0.92 ± 0.08[a,c]
RPF *ml/min*	6.83 ± 0.31	2.55 ± 0.25[a]	4.40 ± 0.37[a,b]	3.89 ± 0.46[a]
FF	0.27 ± 0.01	0.31 ± 0.01	0.28 ± 0.01	0.25 ± 0.02[b]
RVR *mm Hg/(ml/min)*	10.9 ± 0.5	33.0 ± 2.9[a]	15.8 ± 1.6[b]	19.2 ± 2.2[a,b]
LKW *g*	1.46 ± 0.05	5.02 ± 0.33[a]	3.63 ± 0.14[a,b]	4.04 ± 0.18[a,b]
LKW/100 g BW	0.45 ± 0.01	1.52 ± 0.09[a]	1.17 ± 0.05[a,b]	1.33 ± 0.04[a]
% Tubules (cells)	72.0 ± 5.0	20.6 ± 2.4[a]	26.9 ± 2.1[a]	26.0 ± 1.8[a]
% Tubules (lumen)	11.9 ± 2.8	26.9 ± 3.7[a]	25.1 ± 1.4[a]	29.0 ± 1.5[a]
% Tubules (total)	83.9 ± 2.3	47.5 ± 3.9[a]	52.0 ± 2.4[a]	55.1 ± 2.5[a]
% Glomeruli	7.1 ± 1.1	3.1 ± 0.3[a]	3.9 ± 0.2[a]	3.3 ± 0.5[a]
% Vessels	0.1 ± 0.1	0.8 ± 0.4	0.4 ± 0.1	0.5 ± 0.1
% Interstitium	8.8 ± 2.3	48.6 ± 3.9[a]	43.8 ± 2.5[a]	41.0 ± 2.7[a]

Abbreviations are: enal, enalapril; hydral, hydralazine; other abbreviations as in Table 1.

[a] $P < 0.05$ vs. controls

[b] $P < 0.05$ vs. cystic rats

[c] $P < 0.05$ vs. cystic/enal rats

haps by stimulating the RAS (discussed later in this article). Other hormonal mediators that might contribute have received relatively little study. It has been reported in ADPKD patients that plasma endothelin (ET) levels are elevated [24], that ET is present in human cyst fluid [26], and that there is a linear association between ET and blood pressure in hypertensive patients [27]. The intrarenal ET system is also activated in animal models. In the *cpk* mouse model, ET and ET receptor mRNAs increase in parallel with cyst growth [28]. ET levels are also elevated in the Han:SPRD model, and combined ET_A/ET_B receptor antagonism reduces MAP and increases RPF [29]. Studies in animal models have invoked a role for epidermal growth factor [4, 30], which in addition to its growth promoting effects, is known to cause renal vasoconstriction in normal animals [31]. Little is known of the activity of the nitric oxide system in this disease, although recent preliminary reports noted impaired endothelium-dependent vasodilation in ADPKD patients, which was not improved with administration of L-arginine (abstract; Wang et al, *J Am Soc Nephrol*, 8:383A, 1997), and failure of L-arginine to slow the progression of cystic disease in Han:SPRD rats (abstract; Yoshida et al, *J Am Soc Nephrol* 9:385A, 1998).

The RAS is being increasingly recognized to promote renal injury via a number of different mechanisms. The most well known are the typical hemodynamic effects induced by Ang II (hypertension, decreases in renal blood flow more than in GFR, increases in efferent arteriolar resistance, filtration fraction and glomerular capillary pressure, and reduction in the ultrafiltration coefficient). More recently, it has been recognized that Ang II also promotes renal injury by effects on cellular growth, mesangial matrix formation, elaboration of cytokines, and stimulation of interstitial fibrosis [32]. In most models of glomerular disease, the amelioration of abnormal intrarenal hemodynamic derangements is associated with reduction of proteinuria and glomerular sclerosis [33, 34]. More recently, it has been noted that an interruption of the RAS with ACEI or Ang II AT_1 receptor antagonists can slow the progression of diseases, which are more tubulointerstitial in nature, such as chronic puromycin nephrosis [35], cyclosporine nephrotoxicity [36], and chronic allograft rejection [37]. In some of these cases, such as cyclosporine nephrotoxicity [38], there is an apparent dissociation between functional protection (preservation of GFR) and structural protection (limitation of fibrosis).

This dissociation may prove to be the case in ADPKD. Our data in this study confirm previous observations that the RAS plays a prominent role in mediating the renal hemodynamic changes in this model, at least in the earlier phases. Acute administration of the ACEI enalaprilat had a trivial effect on blood pressure, but a profound effect on renal hemodynamics, increasing GFR and RPF by some 50%. Such an exuberant response to RAS inhibition is unusual; even in models in which chronic ACEI is protective, such as experimental diabetes or renal ablation, the acute hemodynamic responses are quite modest [39, 40]. The clearly enhanced renal response to ACEI is quite consistent with the hypothesis that the intrarenal RAS is activated in this model, exerting a strong and tonic influence on renal vascular tone. In analogy, the activation of the intrarenal RAS has similarly been invoked to explain observations of enhanced renal vascular responsiveness to Ang II receptor antagonism in some patients with diabetes, despite the absence of systemic RAS activation [41]. Intrarenal RAS activation has previously also been invoked to explain attenuated Ang II responsiveness in patients with ADPKD [17]. However, such altered responsiveness is not universally found clinically (abstract; Iversen et al, *J Am Soc Nephrol* 8:374A, 1997), and we found equivalent responsiveness to Ang II in both cystic and control rats. Future studies using direct renal intra-arterial infusions to eliminate confounding effects of changes in systemic vascular hemodynamics may prove useful in further defining this issue. Alternatively, the administration of an ACEI is known to influence the levels of both bradykinin and nitric oxide, and therefore, contributions of these mechanisms cannot be excluded as explanations for the effects of enalaprilat.

Despite the clear evidence for a functional role of Ang II, we were unable to biochemically document any activation of the RAS. In fact, PRCs were consistently reduced in cystic rats, even when stimulated by anesthesia and surgery, or in the presence of enalaprilat. Studies in the 40-week rats, which were done under very brief anesthesia, confirmed continuing reduction in PRC and in circulating Ang II levels as well. We were also unable to document activation of the intrarenal RAS using measurements of Ang II. Previous studies in kidneys from ADPKD patients have noted hyperplasia of the juxtaglomerular renin apparatus, with no clear increase in renin-containing cells in that location, but redistribution and expansion of renin immunoreactivity into blood vessels and the tubulointerstitium [42, 43], as well as in the cystic epithelium and the cyst fluid [15]. In contrast, studies in the Han:SPRD model have generally noted suppression of both systemic and intrarenal renin formation [21, 22]. In accordance with the renin findings, we found that renal Ang II levels were similar or possibly reduced in cystic rats as compared with normal controls. These observations notwithstanding, a full understanding of the intrarenal RAS in this disease remains to be developed. Despite the massively enlarged kidneys, the reduction in functioning renal parenchymal mass in the cystic rats may have contributed to the reduced levels seen at the whole kidney level. Our studies did not address the localization of intrarenal Ang II or other renal RAS components. Whether the RAS (its components or receptors) is activated in the cystic kidney or perhaps in specific (for example, vascular) compartments therein awaits further testing. Because Ang II is only one mediator in a panoply of opposing vasoconstrictors and vasodilators, it remains possible that even low levels of RAS activity are important in the absence of appropriate vasodilatory compensation. In any event, the hemodynamic studies clearly indicate an important role for this system in mediating renal hemodynamic function.

We next explored the importance of the RAS in chronic studies, comparing the hemodynamic and structural sequelae of antihypertensive regimens, which suppress (enalapril) and stimulate (hydralazine) the RAS. In glomerular diseases, drugs that block the RAS and ameliorate glomerular hypertension are superior to those agents that do not block that system [44–46]. Our studies with enalapril are similar to those noted in two previous studies of ACEI [22, 23], in which rats were studied at slightly older ages (3 to 5 months). In the studies of Ogborn, Sareen, and Pinette, amelioration of renal growth and serum creatinine were noted with cilazapril, even though the degree of blood pressure reduction was imperfect [23]. In the studies of Keith et al, both enalapril

and the AT_1 receptor antagonist losartan were effective in preserving renal function (as assessed by serum creatinine) and structure (measured as cyst burden) [22]. Assuming that cyst burden correlates with kidney weight, our findings are in agreement. However, those studies did not specifically evaluate the parameter of tubulointerstitial expansion.

Hydralazine (with or without diuretics) has generally been ineffective, although in some models, it appears to be protective early in the course of the disease [44]. Using equi-antihypertensive doses, we found that hydralazine was less effective than enalapril at attenuating renal vasoconstriction at 10 weeks of age. Structurally, both drugs limited renal growth, enalapril slightly more than hydralazine, although neither limited tubulointerstitial expansion. In contrast to our studies, Keith et al did not find equivalent protection with hydralazine [22]. However, in their study, adequate blood pressure control was not achieved in the hydralazine group, and thus, failure to control hypertension may have contributed to the absence of renal protection. Our morphologic studies suggest that in spite of the apparent tonic influence of the RAS, effective reduction of systemic arterial pressure is beneficial in the short term, regardless of specific effects on the RAS. However, the hemodynamic data indicate the enalapril is superior in its hemodynamic protection.

The long-term outcomes of rats treated with enalapril or hydralazine were similar in some parameters and not in others. Renal growth was clearly attenuated with enalapril, but not hydralazine. It is interesting that long-term enalapril was associated with a modest, but significant limitation of somatic growth, an effect we have noted in previous long-term ACEI studies [47]. However, the limitation of renal hypertrophy could not be ascribed solely to this effect because the kidney/body weight ratio was also reduced. Assuming that renal size is largely related to renal cyst burden, then it seems likely that ACEI limited cyst number and/or size, whereas equivalent antihypertensive control with hydralazine did not. Enalapril also afforded specific protection in terms of reducing proteinuria, which is modest but progressive in this model, as well as in limiting heart weight to values similar to those seen in noncystic control rats. Clinically, both renal hypertrophy [11] and proteinuria [48] are risk factors for progression of renal insufficiency; proteinuria appears to correlate with both hypertension [49, 50] and hypertrophy [49]. Accordingly, the beneficial effects of enalapril on both parameters may have contributed to the observed preservation of serum creatinine. It should also be noted that all animals in this study received a normal-sodium diet. Patients with ADPKD exhibit increased total body sodium levels [24], and the rats may as well. Any beneficial effects of enalapril might have been accentuated had the rats also received dietary sodium restriction and/or diuretic therapy. As is the clinical case, the failure to restrict sodium intake may have attenuated the beneficial effects of ACEI.

However, in other parameters, the two drugs were more equivalent. Both attenuated progressive renal insufficiency, as reflected by serum creatinine values. Although serum creatinine is not a sensitive reflection of GFR in the rat, there were clear differences between the treated and untreated rats and clearly no difference among rats receiving antihypertensive drugs. Similarly, morphologic injury, as reflected by expansion of the interstitial area, and attendant fibrosis appeared to be equivalent with the two drugs. The effects on the RAS were predictable. Enalapril increased PRC but reduced blood and renal Ang II levels, whereas hydralazine increased PRC (but not blood Ang II levels) and actually increased left kidney Ang II levels, when expressed per mg renal protein.

In general, the long-term studies support the importance of blood pressure control *per se* but do not unequivocally support the superiority of one antihypertensive regimen over another. Previous studies of ACEI in clinical ADPKD have been disappointing. In contrast to clinical glomerular diseases, ADPKD has not been very amenable to strategies to slow progression using ACEI [48]. In the later stages, ACEI appears to accelerate renal insufficiency in ADPKD [51]. It may well be that the beneficial effects of ACEI are more prominent and promising in the early stages of cyst growth and development, particularly with concurrent sodium restriction. As in most forms of renal disease, advanced structural injury in the later stages may shift the balance toward critical intrarenal dependence on Ang II, where the risks outweigh the benefits of antagonizing the RAS.

Taken together, these data suggest that ACEI may have a superior advantage in modifying some outcomes and risk factors (proteinuria, renal size), but may not confer easily detectable benefit in others (preservation of GFR and limiting development of interstitial fibrosis). Unlike many glomerular diseases, the data would suggest that effective antihypertensive therapy is the key and that the reduction of blood pressure itself is at least as important as the specific choice of antihypertensive regimen.

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